OBSERVATIONS ON THE PRODUCTION OF ANTIBODIES AFTER ANTITYPHOID INOCULATION *

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The use of antityphoid vaccine in armies and among civilians has provided opportunity for numerous observations on antibody-production. The technic used in the study of the typhoid antibodies varies so greatly that in this brief résumé of recorded observations no attempt is made to discuss the detail of methods.

AGGLUTININ

Agglutinin has been studied more frequently than the other immune bodies that follow antityphoid inoculation. Leishman,[1] Wright,[2] Russell,[3] and Whitmore,[4] among others, made extensive series of agglutinin tests on soldiers in order to throw light on immunity against typhoid—on the question of when it appears, its duration, and other problems in connection with it. Burlingame,[5] and Wade and McDaniel[6] made similar studies in large public institutions, while Wallstein,[7] Moon,[8] Reiss,[9] Patrick,[10] Hamilton,[11] Harrison,[12] Thomsen,[13] Felke,[14] Ziersch,[15] Widal,[16] and many others studied agglutination in smaller groups.

The agglutination test has been widely employed in estimating the efficiency of vaccines. Schattstaedt[17] and Castellani[18] worked in this field.

Cummins and Cumming,[19] Dreyer, Walker, and Gibson,[20] Castellani,[21] and others used the agglutination test after typhoid bacilli had been injected in

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[1] Brit. Med. Jour., 1913, 2, p. 499. Jour. Roy. Army Med. Corps, 1907, 8, p. 463; 1909, 12, p. 163.

[2] Studies in Immunization, 1909.

[3] Brit. Med. Jour., 1913, 2, p. 499. Bull. Johns Hopkins Hosp., 1910, 21, p. 83. Boston Med. and Surg. Jour., 1911, 164, p. 1.

[4] Bull. Johns Hopkins Hosp., 1915, 26, p. 88.

[5] Journal-Lancet, 1915, 35, p. 165.

[6] Am. Jour. Pub. Health, 1915, 5, p. 137.

[7] Jour. Exper. Med., 1912, 16, p. 315.

[8] Jour. Am. Med. Assn., 1913, 60, p. 1764. Jour. Infect. Dis., 1914, 14, p. 56.

[9] München. med. Wchnschr., 1915, 62, p. 1278.

[10] Glasgow Med. Jour., 1915, 83, p. 268.

[11] Tr. Chicago Path. Soc., 1912, 8, p. 151.

[12] Jour. Roy. Army Med. Corps, 1907, 8, p. 472.

[13] Hospitalstid., 1915, 58, p. 211.

[14] München. med. Wchnschr., 1915, 62, p. 578.

[15] Ibid., p. 1310.

[16] Presse méd., 1915, 23, p. 305.

[17] Jour. Am. Med. Assn., 1915, 65, p. 1713.

[18] Lancet, 1909, 177, p. 529.

[19] Jour. Roy. Army Med. Corps, 1912, 19, p. 389.

[20] Lancet, 1915, 188, p. 324.

[21] Ibid., 1913, 184, p. 595.

conjunction with other bacteria, to determine whether or not the production of specific typhoid immune bodies is modified by the presence of other bacteria. Sera from persons inoculated in this manner usually agglutinate typhoid bacilli in a dilution equal to, or higher than that employed in sera from a simple typhoid inoculation.

Results obtained in agglutination tests vary. Cahn-Bronner,[22] after studying 104 cases, decided that the 85th day marked a definite dividing line between the high and the low agglutinin content of the blood. Ziersch,[15] on the other hand, stated that the agglutinin content was subject to recognizable laws neither quantitatively nor with respect to time. However, in the literature, I found that new-formed agglutinins are recorded present in fully 90% of inoculated persons. Speaking generally, one may say that they are produced a few days after the first injection, reach their height within 1 or 2 months thereafter, and persist at least 1 year, and frequently 2 years or longer.

OPSONINS

Leishman[23] considers that the phagocytic index is the most reliable test of the value of a particular typhoid vaccine. In estimating opsonins investigators have generally employed (1) the phagocytic index and (2) the point of opsonic extinction. The latter method is considered to be more satisfactory by Russell,[3] Klein,[24] and others, because the poor staining qualities of the typhoid bacilli within the leukocytes, especially if the serum is active, make it difficult to determine an accurate phagocytic index. Klein found results practically identical whether or not the serum was heated. The period of incubation in opsonic tests has varied greatly; Klein, for example, incubating 8 minutes (heated serum), Russell (heated serum) 2 hours. The majority of investigators, among them Hamilton,[11] Klein,[24] Schattstaedt,[17] Russell,[3] and Castellani,[21] found the rise in opsonins to be more rapid than the rise in other antibodies, the titer higher, and the persistence shorter. Hamilton, making daily observations on opsonins, noted a negative phase, enduring from 2 to 4 days, after each injection, then a rapid rise that remained at its height about 7 days, the opsonins persisting for 2 months or longer. In the majority of cases it appears that opsonins are produced from 5 to 8 days after the first inoculation, reach their height in about 1 month, and disappear in from 4 months to 1 year.

BACTERIOLYSIN

Reports on the bacteriolytic power of sera from persons inoculated with typhoid vaccine for typhoid bacilli conflict. Russell,[3] and Cummins and Cumming[19] place little confidence in bacteriolytic tests because of the irregularity of the results, tho Russell concluded that a rise occurs in bacteriolysins, reaching its height 2 months after the first inoculation. Wallstein[7] considers these tests important. Following the method of Stern and Korte,[25] she found in 24 cases examined that the bacteriolytic titer was highest 2 months after the first inoculation and frequently remained above normal 1 year or more. Schattstaedt,[17] in a series of experiments on immunized rabbits, in which he used equal parts of progressive dilutions of serum and a 24-hour typhoid culture, plating after a 15-minute incubation, found that bacteriolysins were at their highest 6 weeks after inoculation and that they persisted for from 4 months to 1 year.

[22] Med. Klin., 1915, 11, p. 964.
[23] Lancet, 1910, 179, p. 885.
[24] Bull. Johns Hopkins Hosp., 1907, 18, p. 245.
[25] Berl. klin. Wchnschr., 1914, 41, p. 213.

Klein, by a modified Neisser method, obtained in a similar experiment the highest bacteriolytic titer 1 month after the first inoculation. After the inoculation of double (typhoid and paratyphoid) and triple (typhoid + 2 strains of paratyphoid) vaccines Widal found typhoid bacteriolysins in about the same quantities as after simple typhoid inoculation.

COMPLEMENT-FIXATION

Fewer observations have been made on complement-fixation than on other immune reactions after typhoid inoculations. Russell[8] found complement-deviation taking place in such cases, Cummins and Cumming[19] not. Widal, using a method similar to the Wassermann method, found inhibition of hemolysis from 8 to 13 days after the first inoculation of typhoid and paratyphoid vaccine. In some cases the reaction remained positive for 150 days. Felke[14] obtained complete hemolysis throughout a series of 39 cases. He concluded that complement-fixation furnishes a means of diagnosing typhoid fever in the inoculated, since the sera of patients with fever inhibited hemolysis, while sera of the inoculated did not. Hage and Korpp-Peterson[20] disagree with Felke; in their tests they obtained complement-fixation 8 days after inoculation. Thomsen[13] reports 1 strongly positive, and 8 weakly positive reactions 10 days after the second typhoid inoculation.

My own observations were made on two healthy men. Each received 3 subcutaneous inoculations of U. S. Army typhoid prophylactic vaccine at 8 and 10-day intervals. The 1st dose was 0.5 c.c. (500 millions), the 2nd and 3rd doses were 1 c.c. (1000 millions) each. No. 1 had a moderately severe local reaction, and slight constitutional symptoms —malaise, headache, slight rise in temperature, etc.—for 24 hours after the 1st and 2nd inoculations. About 3 hours after the 3rd injection a very severe general reaction came on and lasted for from 24 to 48 hours. No. 2 had moderately severe local reactions after each injection, but practically no constitutional symptoms. Blood was collected aseptically from each patient at 2- or 3-day intervals for 5 weeks, then at longer intervals. The sera were withdrawn and inactivated by heating at 56 C. for 30 minutes, and examined for agglutinin, opsonin, bacteriolysin, and complement-fixation bodies.

AGGLUTININ

The microscopic method was used with inactive sera and a killed suspension of a highly agglutinable typhoid strain in normal salt solution. The tubes were incubated at 37 C. for 2 hours, then kept in an ice-box overnight and readings made in the morning.

The serum from No. 1 had no typhoid agglutinins before inoculation. In 3 days after the first injection the titer was 1:40, in 19 days it had reached 1:640, and in 60 days 1:2560, the highest point. Observations were then discontinued.

[20] Deutsch. med. Wchnschr., 1915, 41, p. 1328.

The normal serum from No. 2 agglutinated typhoid bacilli when it was diluted 1:10. In 5 days after the first injection, the titer was 1:40, in 19 days 1:160, and in 63 days 1:5000. Agglutinins then decreased until the 134th day, when the last examination was made, and the titer found to be 1:80. In both cases there was a slight fall in agglutinins after each inoculation.

OPSONINS

Estimation was made by diluting the serum to the point of opsonic extinction. The dilution in which 50 leukocytes had the same percentage of cells taking part in phagocytosis as a normal control with salt solution, was considered the point of extinction. Varying dilutions of inactive sera, human leukocytes, and killed typhoid bacteria were incubated in capillary tubes at 37 C. for 15 minutes. A film was made from each tube, stained, and the number of leukocytes taking part in phagocytosis observed.

In No. 1 there was an almost immediate rise in opsonins, the titer reading 1:30 on the 3rd day, 1:20 on the 5th, and 1:240 on the 10th. There was a slightly negative phase after the second and 3rd inoculations. From the 33rd to the 60th day (marking the last observations), the titer remained constant at 1:240.

In No. 2 there was also an immediate production of opsonins. The height of the opsonic curve, 1:480, was reached on the 21st day after the 1st injection. It remained about 1:240 from the 26th to the 113th day, then fell to 1:120 on the 134th day.

BACTERIOLYSIN

Preliminary tests showed that 0.0125 c.c. of fresh guinea-pig serum, while having in itself little or no bactericidal effect, was sufficient to re-activate a normal inactive serum. One-tenth cubic centimeter of a 1:12 dilution of normal re-activated serum, plated after a 3-hour incubation at 37 C. with 0.02 c.c. of a typhoid suspension, gave approximately sterile plates. The typhoid suspension was of such titer that 0.1 c.c. of 0.02 c.c. typhoid suspension in 0.6 c.c. of broth plated at once gave from 500 to 600 colonies per plate.

In the bacteriolytic test, varying dilutions of each serum were placed in tubes and a fixed amount of fresh guinea-pig serum (0.0125 c.c.) and of typhoid suspension (0.02 c.c.) added to each tube. For each dilution a control tube containing a corresponding amount of inactive serum and of typhoid suspension was set up. Two other controls were also used: one of typhoid bacilli, fresh guinea-pig serum, and broth; the other of typhoid bacilli and broth only. From each of these tubes 0.1 c.c. was plated on agar at once, in order to determine whether or not the colony counts from all the tubes showed the anticipated similarity. The tubes were then incubated 3 hours at 37 C. and again 0.1 c.c. from each was plated. Counts of colonies were made after a 24-hour incubation. The highest dilution of active serum that showed a markedly lower count than the corresponding serum control, was considered to show

the amount of bacteriolysin produced. The plates made at once had from 500 to 600 colonies per plate. After a 3-hour incubation the control plates (typhoid, typhoid + complement, inactive serum + typhoid) contained 1000 or more colonies. The plates from the reactivated serum varied from sterility to 1000 or more colonies per plate.

In both Nos. 1 and 2 the titer of the sera before antityphoid inoculation was 1:48. After each inoculation a slight fall occurred in the bacteriolytic power. The highest titer reached in No. 1 was 1:384, 30 days after the first injection of vaccine. On the 60th day the titer was the same. No. 2 on the 26th day after inoculation had a titer of 1:384. After 74 days the titer decreased to 1:96 on the 113th day and 1:48 on the 134th day.

COMPLEMENT-FIXATION

The complement-fixation tests were made according to the technic employed in the Wassermann test (one-tenth method).

Antigen.—The U. S. Army vaccine was used as antigen. The anti-complementary unit was 0.2 c.c., which remained relatively stable throughout the several months in which the tests were made. Sera from convalescent typhoid patients bound complement in the presence of from 1/16 (0.01 c.c.) to 1/64 (0.005 c.c.) of this unit. Accordingly, in the tests, antigen was varied from 1/2 to 1/64 unit.

Serum.—The sera were inactivated by heating to 56 C. for 30 minutes. Two hundredths cubic centimeter was used in the test. Titration showed no natural antisheep amboceptor to be present in either of the sera. Fresh guinea-pig serum—0.1 c.c. of 1:10 dilution, 2 units—was used as complement.

Serum, antigen, and complement were incubated for 1 hour at 37 C.; then 0.1 c.c. of a 5% suspension of washed sheep corpuscles and 2 units of antisheep amboceptor were added to each tube and the whole incubated at 37 C. for from 15 to 30 minutes according to the controls. The customary controls (antigen, serum and hemolytic) were set up with each test, and serum from a convalescing typhoid patient was used as the positive serum control.

The serum from No. 1 showed slight binding power 14 days after the 1st injection, but was negative after the 3rd injection. It became slightly positive again on the 19th day after the 3rd injection, gradually increasing in binding power until it fixed 1/16 of the anticomplementary unit on the 33rd day. The titer was the same on the 60th day.

The serum from No. 2 began to show binding power on the 17th day after the 1st injection, reaching a maximum on the 33rd day, when it bound 1/32 of the anticomplementary unit. Binding power then decreased until there was complete absence of inhibition on the 124th day.

SUMMARY

Agglutinin appeared in the two men on the 3rd and 5th days respectively, after the 1st inoculation, the highest point being reached in about 60 days; it was present in the serum of Patient 2 on the 134th day.

Opsonin appeared in 3 days and reached its highest concentration in 10 days in No. 1 and in 20 days in No. 2. In No. 1 opsonin was present on the 60th day and in No. 2 on the 134th day. Observations were then discontinued.

Bacteriolysin was increased on the 21st day and in both cases reached the highest point within a week.

Complement-fixation was obtained in No. 1 on the 14th day and was most marked on the 25th day. In No. 2 fixation was obtained on the 17th day and was most marked from the 29th to the 63rd days, after which the power of fixation decreased, and was wholly lost on the 124th day.

There was a slightly negative phase after each inoculation.

The severity of the general reaction in Patient 1 seemed to have no effect on antibody-production.

CONCLUSIONS

After injection of typhoid vaccine into human beings specific antibodies develop in the blood. They reach the highest concentration in from 1 to 2 months, after which they gradually diminish. Opsonin appears to develop earliest. Agglutinin, so far as known now, persists the longest, having been demonstrated to be present 2 years and even longer after the vaccination. Specific complement-fixation is obtainable with the sera of persons injected with typhoid vaccine, hence this test is not distinctive of typhoid fever.